aspects of the differentiation of these neurons need be the result of cell-cell interactions.

Our results suggest that local cell interactions affect neuronal branching. Indirect support for this hypothesis comes from experiments in insects in which patches of integument have been transplanted. Anderson and Bacon (*19*) found that neurons from transplanted wind-sensitive hairs in locusts projected according to their original location. Similarly, Murphey *et al.* (*20*) found that most neurons from transplanted cerci in crickets produced their normal arborizations despite the unusual position of the cerci. Thus, when neurons and their local environments are transplanted together, neuronal differentiation is essentially unaltered.

Other laser ablation studies in *C. elegans* have shown that disruption of cellular interactions results in a range of effects on neuronal development. For example, the absence of neighboring cells prevents the production of the cell lineage that gives rise to the dopamine-containing postdeirid neurons (*21*). In addition, the ventral cord–precursor P1 will produce a motor neuron (VA1) if the more anterior precursor, cell W, is present, but P1 will generate an interneuron (AVF) by the apparently identical lineage if W is absent (*21*). Thus, cell-cell interactions can influence a range of differentiated characteristics: the production of specific cell lineage patterns, the total structure and function of a single cell, or, as in the case of the touch cells, the substructure of a particular neuron.

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Gamma Interferon Synthesis by Human Thymocytes and T Lymphocytes Inhibited by Cyclosporin A

Abstract. *Cyclosporin A depresses the synthesis of gamma interferon by human thymocytes and T lymphocytes in vitro. This observation is of potential clinical significance because the long-term treatment of transplant patients with cyclosporin A, a widely used immunosuppressive agent, can give rise to B-cell lymphoma resulting from Epstein-Barr virus activation.*

Cyclosporin A (CsA), a fungal metabolite of *Trichoderma polysporum* is a cyclic polypeptide containing 11 amino acids (molecular weight, 1206.6). It is an effective immunosuppressant with low myelotoxicty (*1*) and has been used successfully as the primary drug to suppress the rejection of transplants of nonmatched cadaver kidneys, bone marrow transplants, and liver transplants (*2*). One of the risks of allogeneic organ transplants and long-term treatment with immunosuppressive agents is the development of B-cell lymphomas as a result of the activation of Epstein-Barr virus (EBV)-infected B lymphocytes (*3*). Crawford *et al.* (*4*) provided evidence that lymphocytes treated in vitro with CsA are unable to respond to the challenge of EBV-infected B cells and permit their polyclonal proliferation.

Cyclosporin A prevents the response of T lymphocytes to allogens and certain mitogens (*5*) apparently by binding to receptor sites that are in close association with the receptors for concanavalin A (ConA), phytohemagglutinin (PHA), and the histocompatibility antigen DR (*6*). The synthesis of the T-cell growth factor interleukin 2 and the expression of receptors for interleukin 2 on the cell membrane are inhibited by CsA (*5*).

In view of the potential importance of gamma interferon (γ-IFN) in the immune response, we designed a study to investigate whether CsA also inhibits the synthesis of γ-IFN by human T lymphocytes and thymocytes. Studies carried out in our laboratory have shown that γ-IFN increases T-cell–dependent antibody synthesis by B cells (*7*). We also demonstrated that thymocytes, although not yet immunocompetent, can be induced to synthesize γ-IFN in vitro in cultures containing Con A or PHA and B lymphoblastoid cells. The requirement for B lymphoblastoid cells could be replaced by phorbol myristate acetate (PMA). Neither PMA nor B lymphoblastoid cells alone could induce γ-IFN synthesis by human thymocytes (*8*). This is in contrast to T lymphocytes, which can be induced to synthesize γ-IFN with only one inducing agent (*9*). The effect of CsA on human thymocytes has not, to our knowledge, been reported.

We provide evidence that CsA, in concentrations corresponding to those in the serum of patients treated with CsA, inhibits γ-IFN synthesis in vitro by human thymocytes and T lymphocytes. We investigated the role of CsA on thymocyte and T-lymphocyte functions by determining its effect on γ-IFN synthesis induced by a number of agents in vitro and by assessing its effect on cellular proliferation.

Thymocytes were isolated from sections of thymus obtained in the course of cardiac surgery of infants and young children. Isolated thymocytes were washed in medium and immediately cultured with the appropriate inducing agents (*8*). Mononuclear cells, separated by density centrifugation on a Ficoll-Hypaque gradient (density, 1.077 g/cm^3), were depleted of adherent cells by incubation on a plastic surface for 3 hours

and passed through a column containing nylon wool. At the initiation of culture, CsA was added, and thymocytes or T lymphocytes were cultured in 96-well (200 μl) microtiter flat-bottom plates containing RPMI 1640 medium supplemented with 5 percent heat-inactivated fetal calf serum (FCS), 100 units of penicillin, 100 μg of streptomycin, and 0.25 μg of amphotericin B per milliliter. Cultures were incubated in a humidified atmosphere containing 5 percent CO_2 at 37°C. Interferon was determined in the conditioned medium, and the proliferative rate of thymocytes and T lymphocytes was measured by [^{3}H]thymidine incorporation.

The results of the experiments carried out on thymocytes and T lymphocytes are recorded in Table 1. Addition of Con A induced low titers of IFN synthesis; this synthesis was completely abolished by CsA. Thymocytes proliferate spontaneously during the first 48 hours of culture but this proliferation was not increased by Con A and only slightly decreased by CsA (data not shown). At 120 hours, however, thymocytes ceased to proliferate spontaneously but were induced to proliferate by Con A. The late proliferation induced by Con A was inhibited by CsA, as was IFN synthesis.

Irradiated B lymphoblastoid cells did not by themselves induce thymocytes to synthesize IFN and had little effect on the rate of thymocyte proliferation. Addition of both Con A and irradiated B lymphoblastoid cells induced γ-IFN synthesis and markedly increased the proliferation rate at 120 hours. The synthesis of IFN induced by the combined effect of these agents was almost completely inhibited by CsA, and proliferation at 120 hours was reduced by 85 percent.

Although PMA did not induce thymocytes to synthesize IFN, it depressed the early proliferation of thymocytes and increased late proliferation above basal levels; this proliferation was not modified by CsA. In conjunction with Con A, PMA acted as a costimulator of γ-IFN synthesis and also produced a comitogenic effect. Synthesis of γ-IFN was completely abolished by CsA in cultures supplemented with both PMA and Con A, and the proliferation rate was reduced by 95 percent. When used in combination with B lymphoblastoid cells, PMA induced γ-IFN synthesis and acted as a comitogen. Cyclosporin A inhibited IFN synthesis by PMA-treated thymocytes cultured with irradiated B lymphoblastoid cells; however, it decreased the proliferation rate by only 14 percent. This moderate effect on B lymphoblastoid cell–induced proliferation contrasts with the marked inhibitory effect of CsA on proliferation induced by Con A. When all three inducing agents were added to the cultures, maximal γ-IFN synthesis was observed. This synthesis was inhibited by CsA, and the rate of late proliferation was decreased by 64 percent. These observations indicate that IFN synthesis and thymocyte proliferation induced by Con A are markedly depressed by CsA, whereas the effects induced by B lymphoblastoid cells in conjunction with PMA are somewhat more resistant to inhibition by CsA. The effect of CsA was dose-dependent (*10*).

Unlike thymocytes, T cells have a very low proliferation rate during the first 48 hours of culture, but like thymocytes, they responded to the mitogenic effect of the inducing agents by 120 hours. One inducing agent was sufficient to elicit the T-cell response, and CsA decreased the IFN synthesis by T lymphocytes induced with either Con A or PMA.

Overall, the effects of CsA on T cells are similar to those on thymocytes. Although CsA was effective in inhibiting PMA-induced γ-IFN synthesis by T lymphocytes, it inhibited proliferation only slightly. Failure of CsA to inhibit PMA-induced proliferation of T cells was also reported by Palacios (*11*). Abb *et al.* described the depressant effect of CsA on the production of interferon by human peripheral blood leukocytes in vitro (*12*). Our observations indicate that CsA can inhibit at least one event induced by PMA and suggest that proliferation and γ-IFN synthesis are not necessarily linked.

In conclusion, our data show that CsA inhibits the induction of γ-IFN by thymocytes and T lymphocytes. The devel-

Table 1. Gamma interferon synthesis and [^{3}H]thymidine incorporation by human thymocytes and T lymphocytes in the presence (+CsA) and absence (−CsA) of cyclosporin A. Thymocytes were isolated from a thymus specimen in RPMI 1640 medium. Cells were washed and divided into two portions (6×10^6 cells/ml), and phorbol myristate acetate (PMA, 5 ng/ml) was added to one portion. Both portions were incubated in a humidified atmosphere containing 5 percent CO_2 for 3 hours at 37°C. Thymocytes were then cultured for 120 hours in flat-bottom microtiter wells (200 μl) at a density of 6×10^6 cells per milliliter. Concanavalin A (10 μg/ml), irradiated (4800 rad) B lymphoblastoid cells (B_1 cells, 3×10^5 cells per milliliter), and CsA (1000 ng/ml) were added as indicated. [^{3}H]Thymidine incorporation was determined during the last 18 hours of culture by pulsing with 1 μCi of [^{3}H]thymidine per well (specific activity, 6.7 Ci/mmole). The results are expressed in counts per minute per 10^6 cells. The data are given as means ± standard deviation. Interferon titers were determined by microplate assay from antiviral activity in trisomic GM-258 cells, with encephalomyocarditis virus as the challenging virus. A laboratory standard for human γ-IFN was included in each assay; all titers were expressed in actual laboratory units without correction (*8*). The data recorded were obtained from thymocytes from one donor. All determinations were carried out in triplicate, and the experiment is representative of three experiments with thymocytes from different donors. Mononuclear cells were isolated from a plateletpheresis residue by Ficoll-Hypaque density centrifugation (density, 1.077 g/cm^3). Adherent cells were removed by incubation (90 minutes at 37°C) on a plastic surface in complete RPMI 1640 medium. The nonadherent cells were passed through a column of activated nylon wool, and T cells were eluted with complete medium. The T cells were divided into two portions and suspended in complete medium at a density of 6×10^6 cells per milliliter. Phorbol myristate acetate (5 ng/ml) was added to one portion, and both portions were incubated for 3 hours. Conditions for incubation, IFN determination, and [^{3}H]thymidine incorporation were as described above. The experiment was carried out with T cells from one donor and is representative of three experiments.

Additions	Thymocytes				T lymphocytes			
	IFN titer		[^{3}H]Thymidine incorporation		IFN titer		[^{3}H]Thymidine incorporation	
	−CsA	+CsA	−CsA	+CsA	−CsA	+CsA	−CsA	+CsA
None	< 4	< 4	200 ± 20	300 ± 100	16	< 4	4,400 ± 4,00	1,100 ± 50
Con A	64	< 4	21,200 ± 2,000	300 ± 100	1,024	128	9,300 ± 1,000	4,100 ± 150
B_1 cells	16	< 4	2,200 ± 950	800 ± 150	2,048	512	22,800 ± 2,300	14,500 ± 1,400
Con A and B_1 cells	1,024	8	83,600 ± 6,400	13,000 ± 3,000	1,024	1,024	37,000 ± 1,400	25,600 ± 4,500
PMA	< 4	< 4	2,800 ± 250	2,400 ± 1,000	64	16	17,100 ± 3,000	12,100 ± 3,300
PMA and Con A	1,024	< 4	78,200 ± 1,700	4,200 ± 1,000	2,048	16	23,800 ± 4,000	11,300 ± 2,200
PMA and B_1 cells	512	128	42,500 ± 4,400	35,800 ± 3,500	12,288	6,144	9,500 ± 2,500	10,800 ± 200
PMA, Con A, and B_1 cells	4,096	64	72,300 ± 2,800	26,300 ± 3,400	12,288	6,144	27,100 ± 1,500	21,900 ± 2,000

opment of B lymphoma in transplant patients after prolonged treatment with CsA may be causally related to the inhibitory action of CsA on the synthesis of γ-IFN and other immunoregulatory lymphokines produced by T cells.

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Redox Delivery System for Brain-Specific, Sustained Release of Dopamine

Abstract. *Dopamine was transformed into a redox chemical system for delivery to the brain. The lipoidal form allowed penetration of the blood-brain barrier. Oxidative and hydrolytic processes then transformed the delivery form into a quaternary ammonium precursor of dopamine. The quaternary ammonium precursor was rapidly eliminated from the general circulation, whereas that formed in the brain was locked in, thereby providing a significant and sustained brain-specific dopaminergic activity.*

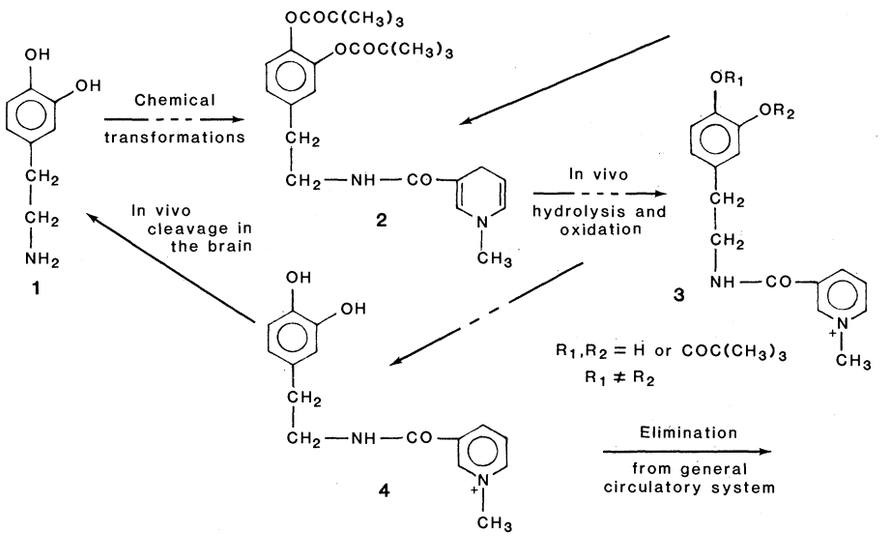
Dopamine (**1**) and related catecholamines do not cross the blood-brain barrier (*1*). Brain delivery of dopamine is critical in the treatment of parkinsonism (*2*), but L-dopa, the best known treatment for this disease, can have undesirable side effects (*3*). Dopamine agonists, used in the treatment of hyperprolactinemia associated with pituitary adenomas or amenorrhea (*4*), also have unwanted side effects. Effective delivery of dopamine itself to the brain would be the most desired way to treat both of these diseases, particularly if a sustained and controlled delivery of dopamine could be provided.

We demonstrated earlier that a redox system of drug delivery based on an interconvertible dihydropyridine ⇆ pyridinium salt carrier (*5*, *6*) can deliver some simple amines, such as phenylethylamine, to the brain in a specific and sustained manner. The drug, when combined with the lipoidal, dihydropyridine carrier, crosses the blood-brain barrier. The carrier is then oxidatively transformed into a charged form, which has the effect of locking in the brain this new carrier-drug combination. The drug is then released in a sustained manner from the charged form. Dopamine, a more complicated molecule than phenylethylamine, requires a more complex delivery system. We now report successful site-specific and sustained delivery of dopamine to the brain, as demonstrated by the presence in the brain of the designed precursor and by the significant and prolonged inhibition of prolactin secretion in vivo.

The chemical delivery system for dopamine is structure **2**, where the amino function of dopamine is connected to the dihydropyridine carrier while the catechol function is protected as the corresponding dipivalyl ester. The brain-specific delivery of dopamine requires a succession of processes, including oxidation of the dihydropyridine ring to the corresponding pyridinium salt (for example, structure **3**), which provides the basis for the locking-in of the molecule in the brain, hydrolysis of the pivalyl esters (**4**) possibly via the 3- and 4-monopivalyl esters, and the release of dopamine (**1**) from **4**, which can be either by hydrolysis or by a reductive process. The possibility of a reductive release of dopamine was suggested by a model for a presynaptic terminal (*7*).

To evaluate this dopamine delivery system, we administered compound **2** intravenously to rats and evaluated the concentrations of the dopamine precursor **4** in the brain and plasma along with concentrations of dopamine in the brain.

After a single intravenous injection of the delivery system represented by structure **2**, compound **4** was effectively locked in the brain and quickly eliminated from the rest of the body (Fig. 1). No 3-*O*-methylated derivative of **4** could be detected in the brain, indicating that these amides are not good substrates for catechol *O*-methyl transferase (COMT). No dramatic increase was observed,



The brain-specific delivery of dopamine (**1**). Compound **2** is the chemical delivery system and **4** is the precursor locked in the brain and eliminated quickly from the rest of the body. Structure **3** depicts the intermediates formed during the stepwise hydrolysis and oxidation processes.